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Metabolic and Structural Studies on Serum- and Liver-Glycosaminoglycans in Normal and Liver-injured Rats

By *A. M. Gressner, W. Köster-Eiserfunke, E. Van de Leur* and *H. Greiling*

Department of Clinical Chemistry and Pathobiochemistry of the Medical Faculty of the RWTH Aachen, FRG

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Summary: The incorporation of [^{35}S]sulfate into total and specific types of serum glycosaminoglycans was studied in rats with acute, subacute or chronic liver injury (liver cirrhosis), and compared with that of normal rats. The macromolecular (protein-bound) nature of serum glycosaminoglycans in normal and diseased animals was also analysed.

The results show a strong increase in rate and extent of [^{35}S]sulfate incorporation into total serum glycosaminoglycans for acutely but a decrease for subacutely and chronically liver damaged rats. The time-course of distribution of label between serum chondroitin sulfate and dermatan sulfate exhibits significant changes in liver-injured animals, in particular a relatively high proportion of dermatan [^{35}S]sulfate in rats with cirrhotic livers.

In comparison with serum glycosaminoglycans the labeling profile of glycosaminoglycans in the cirrhotic liver was quite different (heparan sulfate:dermatan sulfate:chondroitin sulfate = 1:0.34:0.09) and changed only insignificantly during a 1 h labeling period.

The protein-bound moiety of serum glycosaminoglycans was not affected by liver disease; but the elution profile of chondroitin [^{35}S]sulfate from Dowex 1 × 2 for treated rats was altered, thus indicating a structural modification of its carbohydrate chain.

Metabolische und strukturelle Untersuchungen der Glykosaminoglykane im Serum, im Vergleich zur Leber, bei normalen und leber-geschädigten Ratten

Zusammenfassung: Der Einbau von [^{35}S]Sulfat in die gesamten und spezifischen Typen der Glykosaminoglykane im Serum von Ratten mit akuter, subakuter oder chronischer (Lebercirrhose) Leberschädigung wurde untersucht und mit dem gesunder Tiere verglichen. Zusätzlich wurden Untersuchungen zur makromolekularen, insbesondere zur proteingebundenen Struktur der Glykosaminoglykane im Serum normaler und lebergeschädigter Ratten durchgeführt.

Die Ergebnisse zeigen einen starken Anstieg der Rate und des Ausmaßes der Sulfatinkorporation in die gesamten Glykosaminoglykane im Serum bei akut, eine Erniedrigung jedoch bei subakut und chronisch lebergeschädigten Ratten. Der zeitliche Verlauf der Verteilung der [^{35}S]Markierung zwischen Chondroitinsulfat und Dermatansulfat im Serum ließ signifikante Veränderungen bei lebergeschädigten Tieren erkennen, insbesondere einen relativ hohen Anteil von Dermatan [^{35}S]sulfat bei Ratten mit Lebercirrhose.

Im Vergleich zu Glykosaminoglykanen im Serum war das Markierungsprofil der Glykosaminoglykane in cirrhotischer Leber sehr verschieden (Heparansulfat:Dermatansulfat:Chondroitinsulfat = 1:0.34:0.09) und veränderte sich während einer 1-stündigen Markierungsperiode nur geringfügig.

Die proteingebundene Struktur der Glykosaminoglykane im Serum wurde durch experimentelle Lebererkrankungen nicht beeinflußt, jedoch weist die Änderung des Elutionsprofils von Chondroitin[^{35}S]sulfat an Dowex 1 × 2 auf strukturelle Veränderungen seiner Kohlenhydratkette bei Leberschädigung hin.

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Introduction

Previous studies on the metabolism of hepatic glycosaminoglycans have shown that rat liver slices synthesize predominantly heparan sulfate and to a much smaller degree chondroitin sulfate, hyaluronic acid and a keratan sulfate-like fraction (1, 2). Both normal and damaged liver parenchyma, however, seem to be unable to incorporate[^{14}C]amino sugar into dermatan sulfate (1–3), although the latter is present in normal liver and shows a high accumulation in injured liver tissue (4–8).

Consequently the hypothesis was put forward that dermatan sulfate is synthesized in extrahepatic tissues, secreted and transported in the serum and taken up by the liver (3, 9). Because such a metabolic pathway would be of great importance for the pathogenesis of the fibrotic transition of chronically injured liver tissue, we studied the metabolism of total and specific types of sulfated glycosaminoglycans in serum of normal and liver-injured rats, compared it with that in liver. In addition, we investigated the structure of the macromolecular, in particular protein-bound nature of serum glycosaminoglycans.

Materials and Methods

Materials

Chondroitin AC (EC 4.2.2.5) and ABC (EC 4.2.2.4) lyases were obtained from Seikagaku Kogyo Comp., Tokyo, Japan; papain (EC 3.4.22.2, crystalline suspension, 30 U/mg protein) was from Boehringer Mannheim GmbH, Germany; thioacetamide p.a. was from Merck AG, Darmstadt, Germany; *D*-[1-^{14}C]glucosamine hydrochloride (288.6 GBq/mol) and sodium [^{35}S]sulfate (carrier free) were from New England Nuclear Corp., Boston, USA; Sephadex G-100 and Sepharose CL-4B were from Pharmacia, Uppsala, Sweden; Dowex 1 × 2 and Bio-Gel P 2 were from BioRad Laboratories, Munich, Germany.

Treatment of rats

Male Sprague-Dawley rats (250–320 g, Zentralinstitut für Versuchstiere, Hannover, FRG) received food and water ad libitum. Acute liver injury was induced by two repeated intraperitoneal thioacetamide injections of 100 mg/kg in a 24 h interval. One day after the last injection the rats were sacrificed. Subacute and chronic liver injury was produced by oral administration of thioacetamide for 4 weeks and 10 months, respectively, as described previously (2).

In general, each value represents the mean of two to four independent experiments.

Determination of the incorporation of isotopes into total glycosaminoglycans of serum

Rats were injected intraperitoneally with either 37 MBq of sodium [^{35}S]sulfate or 1.3 MBq of [^{14}C]glucosamine hydrochloride and decapitated at various times thereafter. 1–2 ml of blood was collected from the neck vessels and non-hemolytic serum produced. The serum proteins were precipitated by addition of 5 vol. of acetone, centrifuged for 5 min at 3000 *g* and delipidized by washing consecutively with 5 vol. of acetone, chloroform-methanol (volumes, 200 ml + 100 ml) and ethanol-ether (volumes, 300 ml + 100 ml). The final sediment was dried at 60 °C, homogenized in 3 ml of papain-buffer (10) from which an aliquot was taken for the determination of protein (11) and proteolysed for 24 h by the addition of 300 µl (90 U) of papain (1). Total glycosaminoglycans were isolated from the proteolysate (1), dissolved in water and an aliquot was taken for the determination of [^{14}C]-or[^{35}S]-radioactivity in a liquid scintillation spectrometer with an efficiency of 81%.

Determination of the incorporation of isotopes into total glycosaminoglycans of liver

After injection of the isotope the liver was quickly removed, chilled and minced in ice-cold buffer (0.05 mol/l Tris-HCl, pH 7.6 (20 °C), 0.08 mol/l KCl, 0.0125 mol/l $MgCl_2$), freed carefully of blood and homogenized at 2 °C. The protein was precipitated with 5 vol. of acetone and defatted and proteolysed as above. Glycosaminoglycan-associated radioactivity was isolated and counted as described for serum.

Determination of the incorporation of isotopes into specific types of glycosaminoglycans of serum and liver

As described elsewhere (1) total glycosaminoglycans were subjected to enzymatic analysis with chondroitin ABC and AC lyases to determine the incorporation of label into chondroitin sulfate and dermatan sulfate, and degraded by nitrous acid to quantitate the incorporation into heparan sulfate and heparin. The incorporation of radioactivity into specific types of glycosaminoglycans was expressed as percentage of the activity of the unfractionated glycosaminoglycans.

Total labeled glycosaminoglycans of serum were chromatographed on a column (0.6 × 13 cm) of Dowex 1 × 2 (Cl^-) by stepwise elution with an increasing molarity of NaCl (1, 12). The fractions were dialyzed, dried, dissolved in water and subjected to enzymatic and chemical identification.

Gel chromatography of serum glycosaminoglycans

13 ml of serum from normal rats, which received [^{35}S]sulfate 5 h prior to decapitation, were applied to a column (1.4 × 60 cm) of Bio-Gel P 2 and eluted with high ionic strength buffer A (0.02 mol/l Tris-HCl, pH 7.6 (20 °C), 0.5 mol/l KCl, 0.003 mol/l $MgCl_2$). The excluded radioactivity was concentrated by ultrafiltration (filter UM 2, Amicon GmbH) and rechromatographed on a column (2 × 120 cm) of Sepharose CL-4B. The activity eluted with buffer A between 300 and 325 ml was pooled, dialyzed and divided in two equal portions. One portion was rechromatographed on a column (2 × 120 cm) of Sephadex G-100 after proteolytic digestion with 200 µl (60 U) of papain for 48 h at 60 °C, the other one was treated identically but without addition of papain. The eluates were fractionated into 5 ml portions and analysed for radioactivity. All chromatographic procedures were performed at 4 °C.

Density gradient centrifugation of serum glycosaminoglycans

The undigested radioactive material eluted with buffer A between 125 and 150 ml from Sephadex G-100 was concentrated by ultrafiltration (filter PM 10) and adjusted to a starting density of 1.55 kg/l by the addition of solid $CsCl_2$. The gradients (13 ml) were centrifuged for 60 h at 155 000 *g* and 20 °C and then fractionated into 1 ml portions in which the density and radioactivity were determined. The fraction containing the peak activity was proteolysed and subjected to enzymatic analysis for chondroitin sulfate.

Electrophoresis of [^{35}S]-labeled serum components

Serum was obtained from normal rats which recived [^{35}S]-sulfate 5 h before exitus. The proteins were separated by cellulose acetate foil electrophoresis following standard clinical laboratory procedures (13). The bands of more than 20 electropherograms were cut out, pooled and counted for radioactivity after addition of Instagel (Packard).

Results

Labeling pattern of specific glycosaminoglycans in liver and serum

As previously reported (1, 2) rat liver explants do not incorporate [^{14}C]hexosamine and only a very small fraction of [^{35}S]sulfate (0.007–0.013 of total glycosaminoglycans) into dermatan sulfate. The data shown in table 1 confirm the inability of rat liver to incorporate [^{14}C]hexosamine into dermatan sulfate in vivo during a 1 h labeling period. However, using [^{35}S]sulfate as a precursor a fraction of about 0.15 of the total glycosaminoglycan radioactivity was found in dermatan sulfate. Both isotopes were incorporated predominantly into heparan sulfate (0.70 to 0.85) the remainder being recovered in chondroitin sulfate (tab. 1).

The proportions did not change significantly in acutely injured liver (data not shown).

In serum of normal and liver-damaged rats [^{14}C]-glucosamine was incorporated exclusively into chondroitin sulfate. This type of serum glycosaminoglycan was also predominantly labeled after injection of [^{35}S]sulfate. Only a small fraction of [^{35}S]label was found in serum dermatan sulfate and heparan sulfate (tab. 1).

Time course of [^{35}S]sulfate incorporation into serum glycosaminoglycans of normal and liver-damaged rats

Only [^{35}S]sulfate is incorporated both into liver and serum dermatan sulfate. Consequently this type of isotopic precursor was used in the following studies. [^{35}S]sulfate is incorporated very rapidly into serum glycosaminoglycans of normal rats (fig. 1). Nearly a fraction of 0.50 of the final radioactivity of serum glycosaminoglycans (at 300 min) is reached within 45 min after application of the isotope.

Tab. 1. The pattern of incorporation of [^{14}C]glucosamine and [^{35}S]sulfate into specific types of glycosaminoglycans in liver and serum of normal rats. Rats received the isotopes 1 h before sacrifice. Glycosaminoglycans isolated from liver and serum were characterized by enzymatic and chemical procedures. The radioactivity incorporated into specific types is expressed as fraction of total glycosaminoglycan-radioactivity. The ratios are the mean of triplicate experiments.

Isotopic precursor		Heparan sulfate	Chondroitin sulfate	Dermatan sulfate
[^{14}C]Glucosamine	Liver	0.856	0.123	–
	Serum	–	1.00	–
[^{35}S]Sulfate	Liver	0.725	0.042	0.152
	Serum	0.044	0.85	0.065

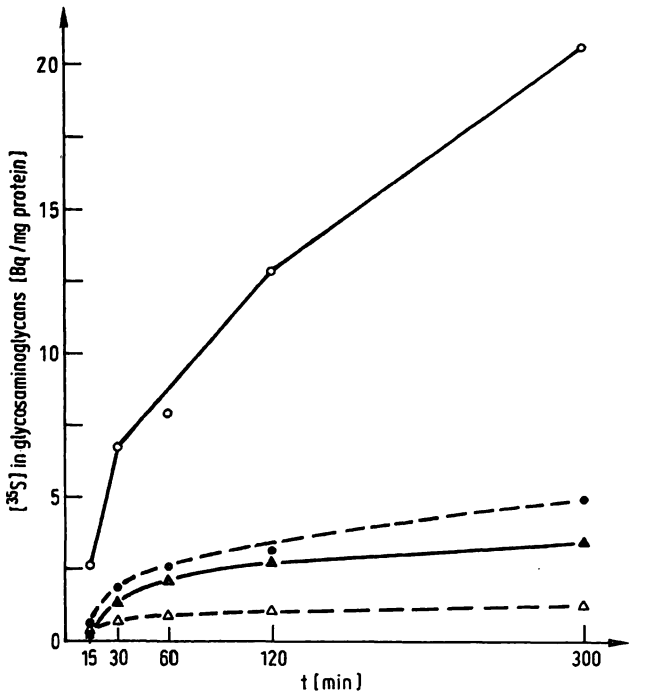

Fig. 1. Time course of [^{35}S]sulfate incorporation into total serum glycosaminoglycans of normal (●– – –●), acutely (○——○), subacutely (△– – –△) and chronically (▲——▲) liver-injured rats.
The animals were sacrificed at various times after injection of the isotope, and the radioactivity incorporated into total serum glycosaminoglycans was determined and referred to the concentration of serum protein. The total serum protein concentration did not change under treatment.

Rate and extent of incorporation of sulfate into glycosaminoglycans are significantly augmented in acutely liver-damaged rats. 5 h after application of the isotope the activity of total serum glycosaminoglycans was over 4 times higher than normal (fig. 1).

In contrast, long- und middle-term liver damage resulted in a 30% and 75% reduction of the respective final activities of serum glycosaminoglycans (fig. 1).

The differences in the kinetics of sulfate incorporation into serum glycosaminoglycans of rats in different stages of liver injury become more complex if the time-dependency of the labeling pattern of specific types of glycosaminoglycans is analysed (fig. 2). In normal rats a fraction of 0.65 of the sulfate incorporated within 15 min into serum glycosaminoglycans was found in chondroitin sulfate, the remainder being associated with dermatan sulfate. Concomitantly with a further time-dependent increase in the fraction of chondroitin [^{35}S]sulfate, the dermatan[^{35}S]sulfate disappears within 2 h from the circulation (fig. 2a). No other sulfated glycosaminoglycans could be detected in serum in significant amounts.

In rats with acute liver injury the proportion of initially [^{35}S]labeled chondroitin sulfate was higher (0.81) where-

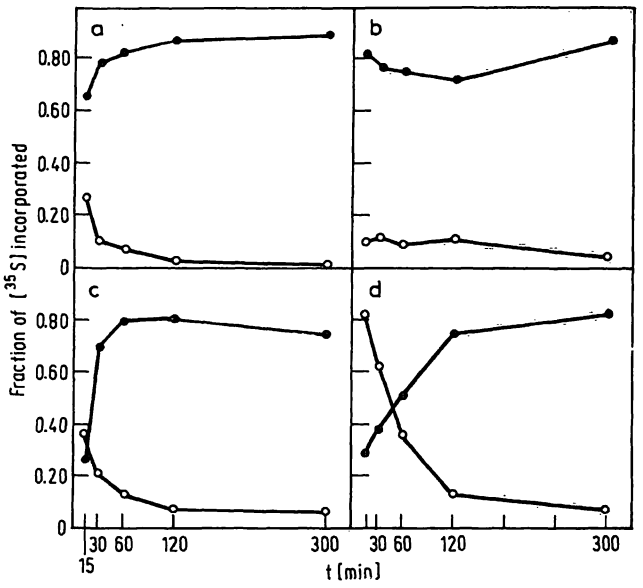

Fig. 2. Time course of the relative incorporation of [^{35}S]sulfate into specific types of serum glycosaminoglycans of normal (a), acutely (b), subacutely (c) and chronically (d) liver-injured rats: The animals were treated as described in fig. 1, individual glycosaminoglycans were identified by chemical and enzymatic procedures and expressed as a fraction of total glycosaminoglycans. Chondroitin sulfate •——•, dermatan sulfate o——o.

as that of dermatan sulfate was lower (about 0.10) than normal. During a 5 h period the relative distribution did not change significantly (fig. 2b).

In animals with subacute and chronic liver damage the initial sulfation pattern was reversed, i.e. the fraction of labeled dermatan sulfate was greater than that of chondroitin sulfate (fig. 2c, d). In long-term liver-injured rats a fraction of 0.82 of [^{35}S]sulfate incorporated into total glycosaminoglycans was found initially (at 15 min) in dermatan sulfate, the remainder in chondroitin sulfate (fig. 2D). Since the fraction of circulating [^{35}S]dermatan sulfate declines more slowly in rats with chronic liver damage than in untreated animals, their relative level of serum dermatan[^{35}S]sulfate was found to be significantly elevated.

Time course of [^{35}S]sulfate incorporation into liver glycosaminoglycans

The kinetics of sulfation of glycosaminoglycans in serum was studied in comparison to that in liver of injured rats. Figure 3a demonstrates a highly active sulfation of glycosaminoglycans in liver which is detectable as early as 2 min after application of the isotope. In contrast, the sulfation of glycosaminoglycans in 1–2 ml of serum (protein concentration about 70 g/l) was not detectable until 10 min after injection of the label. At this time the activity (Bq/mg protein) of total glycosaminoglycans in the liver was about 125 times higher than in serum (tab. 2).

Tab. 2. Ratio of incorporation of [^{35}S]sulfate into glycosaminoglycans of liver and serum of chronically liver-damaged rats. The calculation is based on the data given in fig. 3. The activity of glycosaminoglycans is expressed per mg of protein of liver or serum, respectively.

Time after application of [^{35}S]sulfate	Liver/serum ratios of radioactivity (Bq/mg protein) incorporated into glycosaminoglycans		
(min)	Total	Chondroitin sulfate	Dermatan sulfate
10	127	–	–
15	63	26	16
30	42	6	24
60	74	2	45

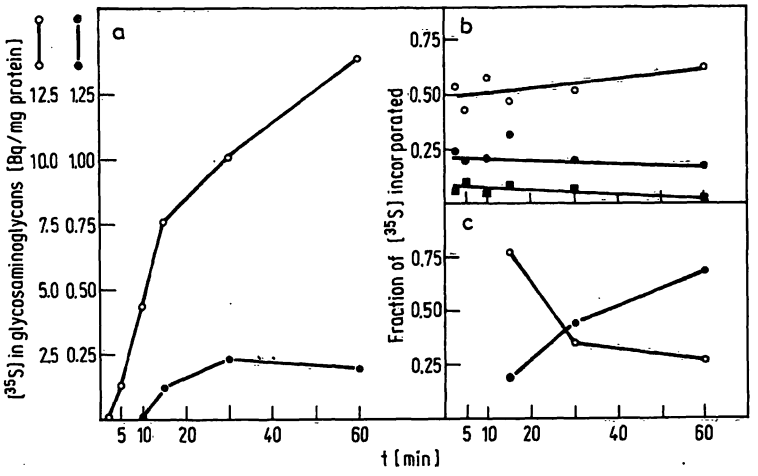

Fig. 3. Time course of [^{35}S]sulfate incorporation into total and specific types of glycosaminoglycans in liver and serum of chronically injured rats. The animals were injected with 18 MBq of [^{35}S]sulfate and decapitated at the times indicated. The radioactivity incorporated into total glycosaminoglycans (a) of serum (•——•) and liver (o——o) was determined. The incorporation into specific glycosaminoglycans of liver (b, o——o heparan sulfate, •——• dermatan sulfate, ▪——▪ chondroitin sulfate) and serum (c, o——o dermatan sulfate, •——• chondrotin sulfate) is expressed as a fraction of the incorporation into total glycosamino glycans.

As illustrated in figure 3b and c the time courses of sulfation of individual glycosaminoglycans in liver and serum of chronically liver-injured rats are strikingly different. The ratios of incorporation of [^{35}S]sulfate into heparan sulfate, dermatan sulfate and chondroitin sulfate of liver were 1:0.34:0.09 and remained nearly unchanged with time. In serum, however, the relative distribution of [^{35}S]sulfate between chondroitin sulfate and dermatan sulfate reversed with time (fig. 2d, 3c).

The ratios of sulfation of liver- and serum-derived dermatan sulfate and chondroitin sulfate were calculated. The data summarized in table 2 indicate a much higher incorporation of sulfate into the glycosaminoglycan types of liver at any time. The liver/serum ratios exhibit a time-dependent increase for dermatan sulfate but a decrease for chondroitin sulfate.

Proteoglycan character of serum glycosaminoglycans

Results presented above demonstrate metabolic changes of serum glycosaminoglycans in rats with experimental liver injury. Further studies were performed to analyse the macromolecular and protein-bound nature of serum glycosaminoglycans and its possible alteration in liver-damaged animals.

Figure 4 shows that the peak [^{35}S]sulfate activity incorporated into serum components comigrates with the α_1-globulin fraction regardless of the origin of the serum, i.e. from healthy or liver-damaged rats.

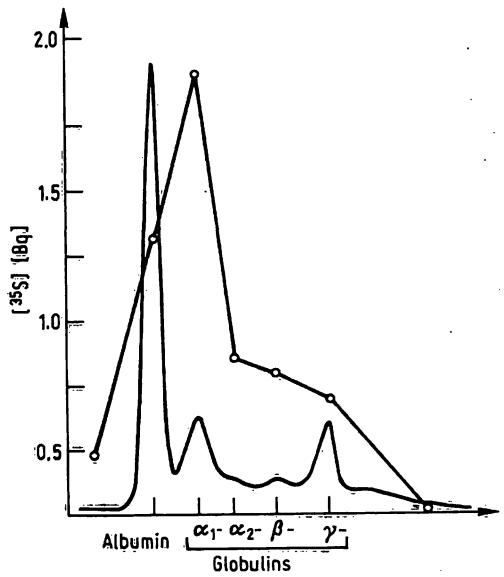

Fig. 4. Cellulose acetate foil electrophoresis of [^{35}S]-labeled components from rat serum. 5 h after i.p. injection of [^{35}S] sulfate into a normal rat, blood was collected and about 50 µl of serum electrophoretically separated. The protein fractions of about 20 electrophoreses were cut out and their radioactivity (o——o) determined. A typical densitograph (——) is illustrated. Essentially identical results were obtained with serum from liver-injured rats (not shown).

However, it is by no means certain that some if any of the activity of the α_1-globulin is proteoglycan in nature. Therefore serum obtained from normal rats injected previously with [^{35}S] sulfate was chromatographed on Bio-Gel P2 to remove low molecular weight [^{35}S]sulfate labeled components (fig. 5). The excluded radioactivity was rechromatographed on Sepharose CL-4B. The fractions which contained chondroitin [^{35}S]sulfate (Kav = 0.76) were further chromatographed on a column of Sephadex G-100 before (fig. 5c) and after (fig. 5d) proteolysis by papain. Extensive degradation to low

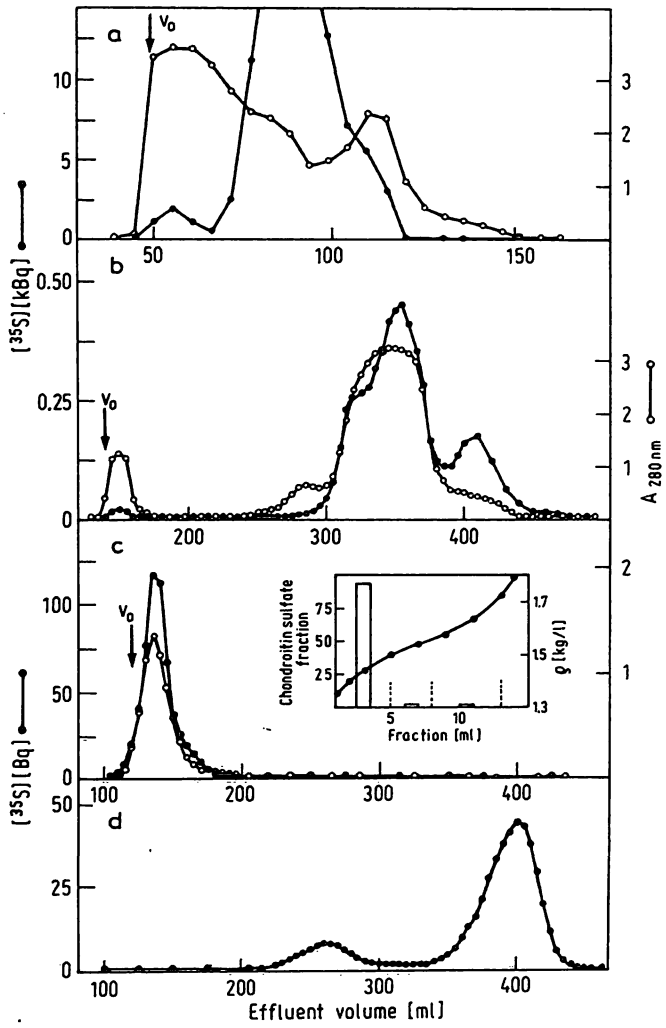

Fig. 5. Gel chromatography of [^{35}S]labeled glycosaminoglycans from normal rat serum.
The animals were decapitated 5 h after injection of [^{35}S]sulfate and blood was collected. 13 ml of serum was applied to a column of Biogel P2 and eluted with buffer A (0.02 mol/l Tris-HCl, pH 7.6, 0.5 mol/l KCl, 0.003 mol/l $MgCl_2$). The activity eluted between 50 and 70 ml (a) was rechromatographed on a column of sepharose CL – 4B in buffer A (b). The fractions between 300 and 325 ml effluent were pooled and divided in two equal portions (a and b). Portion a was digested with papain prior to chromatography on Sephadex G 100 (d); portion b was treated identically but without addition of papain (c). The inset in c represents the distribution of the undigested chondroitin sulfate in 13 ml of a CsCl-density gradient (•——•, density).

molecular weight fractions was observed after proteolytic treatment. The elution pattern of the undegraded chondroitin sulfate (Kav = 0.05) (fig. 5 c) and degraded chondroitin sulfate (Kav = 0.55) (fig. 5 d) was not changed by the addition of excessive amounts of unlabeled chondroitin sulfate and rat serum protein, respectively. It should be emphasized that after proteolysis a major radioactive peak of unknown identity occurred at V_t (380 ml) of Sephadex G-100 (fig. 5 d). The high molecular weight fraction of serum glycosaminoglycans, which was almost excluded from Sephadex G-100 (fig. 5 c), was further characterized by isopycnic density gradient centrifugation under both associating and dissociating conditions (in the presence of 4 mol/l of guanidinium hydrochloride). In each case a fraction of about 0.90 of the total chondroitin sulfate banded near the top of the gradient at a density of d = 1.44 kg/l (inset in fig. 5 c). Neither the gel chromatographic elution pattern of serum glycosaminoglycans nor their distribution in isopycnic gradients showed any change under the influence of liver injury (results not shown).

However, differences were observed in the ion-exchange chromatographic behaviour of [^{35}S]glycosaminoglycans from serum of normal and acutely liver-injured rats. [^{35}S]labeled chondroitin sulfate from treated rats tended to elute at lower ionic strength (0.5 mol/l NaCl) from Dowex 1 X 2 than the glycosaminoglycans isolated from normal rat serum (fig. 6).

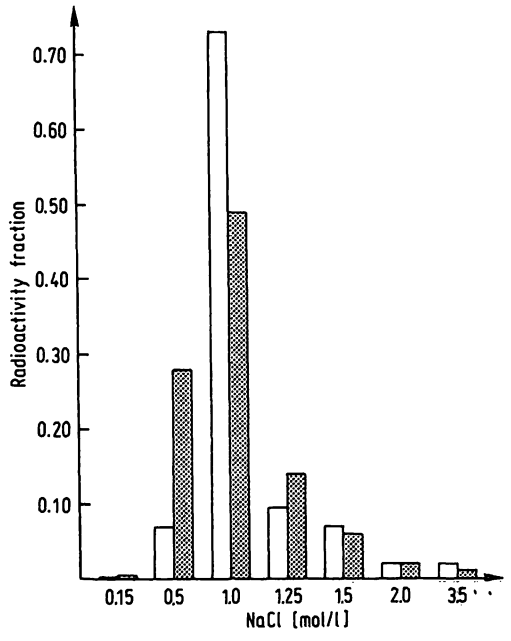

Fig. 6. Anion-exchange chromatography of [^{35}S]-labeled glycosaminoglycans from serum of normal (open bars) and acutely liver-injured (hatched bars) rats.
The animals were decapitated 5 h after injection of [^{35}S]sulfate. Total glycosaminoglycans (containing predominantly chondroitin sulfate) were isolated from serum, applied to a column of Dowex 1 X 2 and eluted with a stepwise gradient of increasing molarity of NaCl. Fractions were assayed for radioactivity.

Discussion

Although distribution and chemical composition of acidic glycosaminoglycans in blood have been reported by several investigators (14–21) detailed information on their physiological functions, metabolism and pathobiochemical alterations are still lacking. It has been reported that human serum contains a preponderance of undersulfated chondroitin 4-sulfate and chondroitin 6-sulfate (21–23) and only a small amount of chondroitinase-resistant acidic glycosaminoglycans (heparan sulfate, dermatan sulfate, keratan sulfate) (20–22). Accordingly, we observed in normal rats a nearly exclusive incorporation of [^{35}S]sulfate and [^{14}C]glucosamine into chondroitin sulfate. The source of serum glycosaminoglycans is unknown but it was suggested that they may derive at least in part from leukocytes or platelets (21, 23–25) or orginate from the extracellular matrices of various tissues. The sites of degradation (e.g. desulfation) of the different types of serum glycosaminoglycans are also not known but obviously glycosaminoglycan-degrading tissues are widespread in the body (26). Several studies indicate that the liver might have an important function in this process (26, 27).

Information on the concentration, composition and metabolism of glycosaminoglycans in serum of patients with liver disease or in serum of experimentally liver-injured rats is lacking. But from the increase of the urinary excretion of glycosaminoglycans in patients with chronic hepatitis or florid cirrhosis (28) concomitant alterations of the concentration and composition of glycosaminoglycans in serum can be deduced. Further studies are necessary to prove such changes and to elucidate their role in the pathogenesis of liver fibrosis.

Slices of normal and injured liver do not incorporate [^{14}C]hexosamine and far less than a fraction of 0.01 of [^{35}S]sulfate into dermatan sulfate (1, 2, 29). It might be possible that an as yet unidentified extrahepatic factor which promotes specifically the synthesis or sulfation of dermatan sulfate in liver is lacking in vitro, but it also seems likely that hepatic dermatan sulfate is supplied to the liver from extrahepatic sources. The present results indirectly support this assumption, since in vivo a fraction of about 0.15 of total liver [^{35}S]glycosaminoglycan activity is located in dermatan sulfate; also significant changes of the kinetics of sulfation of serum dermatan sulfate occur in chronically liver-injured rats, and the liver/serum ratio of dermatan [^{35}S]sulfate increases with time nearly 3-fold, whereas that of chondroitin sulfate and of total glycosaminoglycan declines (tab. 2). However, the specificity of these serum changes for liver injury remains to be determined. From the data given in figure 3 the amount of labeled dermatan sulfate in the whole liver and in the total serum volume of a rat (320 g body wt.) can be calculated, based on a rat plasma volume of 39 ml/kg body weight (30), a serum protein con-

centration of 70 g/l and a total liver protein of 1.1 g. Accordingly 15, 30 and 60 min after injection of [^{35}S]-sulfate the respective total amounts of dermatan [^{35}S]-sulfate in the liver are 20-, 30-, and 57-fold, greater than in the whole body serum. This indicates that either serum dermatan sulfate cannot be the single source of liver dermatan sulfate or that dermatan sulfate coming from the serum is rapidly sulfated in the liver. Since neither liver – nor serum – dermatan sulfate incorporated [^{14}C]hexosamine, its sulfate groups must turn over more rapidly than, and independently from, the carbohydrate chain. Thus incorporation of sulfate in this type of glycosaminoglycan does not represent "de novo" synthesis, but sulfation of an already existing carbohydrate acceptor.

The experimental evidence for a supply of the carbohydrate chain acceptor to the liver via the systemic circulation, as suggested here, is rather circumstantial. Studies on the uptake and deposition of proteodermatan sulfate in normal and diseased liver, where it cannot be degraded by lysosomal enzymes (31, 32), are difficult at present, because homologous liver or serum proteodermatan sulfate is not available in amounts sufficient for intravenous injections or application to the medium of isolated perfused rat livers. The use of a glycosaminoglycan instead of native proteoglycan for such studies might lead to erroneous results since the metabolism of both types is quite different if injected intravenously (33, 34).

In the present study the macromolecular nature of serum glycosaminoglycans was shown, which confirms the results of earlier reports (20, 21, 23, 35). Although glycosaminoglycans can interact electrostatically with a number of serum proteins (36) a covalent linkage to protein (core protein) seems likely since chondroitin sulfate radioactivity could not be displaced from protein by high amounts of the unlabeled counterpart (fig. 5 c). Furthermore, the readdition of serum protein to the glycosaminoglycan obtained after proteolysis was without effect on their gel chromatographic elution pattern (fig. 5 d). The large amount of low molecular weight [^{35}S] labeled material liberated by proteolysis (eluting at V_t, fig. 5 d) was not characterized further. It might partially originate from [^{35}S]sulfate entrapped by undegraded serum proteins, although chromatography was performed in buffers of high ionic strength. The material, however, might also derive from [^{35}S] containing oligosaccharides covalently linked to serum proteins. It is noteworthy that liver damage does not affect the macromolecular composition of serum glycosaminoglycans, but some changes in the carbohydrate chain occur as indicated by the differences in the ion exchange chromatographic elution profiles (fig. 6).

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Priv.-Doz. Dr. Axel M. Gressner
Abteilung Klinische Chemie und Pathobiochemie
der Medizinischen Fakultät der RWTH Aachen
Goethestr. 27–29
D-5100 Aachen